DCode™ Control Reagent Kit for SSCP

Instruction Manual

Catalog Number
170-9151

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4100103 Rev D

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* The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffman-La Roche.

Section 1 Introduction

The DCode control kit for SSCP (Single Stranded Conformational Polymorphism) provides reagents that are used to prepare mutant and wild-type DNA for gel electrophoresis with the DCode universal mutation detection system. The SSCP technique is based on the fact that single-stranded DNA has a defined secondary structure. Sequence differences as small as a single base change can affect this secondary structure and can be detected by electrophoresis in a nondenaturing polyacrylamide gel.[1] Double stranded mutant and wild-type samples are first denatured into single strands and then loaded onto the gel. Differences in mobility of the single strands between the wild-type DNA and the other samples indicate a mutation. SSCP is a widely used mutation screening method because of its simplicity.

The DCode control kit for SSCP contains wild-type DNA, mutant DNA, two 19 base-pair oligonucleotide primers, and 2x SSCP gel loading dye. The mutant DNA is identical to the wild-type DNA, except for a single A to T mutation at fragment base number 110. The PCR reaction will produce a 299 bp fragment. There are sufficient amounts of DNA and primers for five 100 µl PCR reactions for both the mutant and wild-type DNA.

1.1 Kit Components

Item	Concentration	Amount	Volume
Mutant DNA	50 pg/µl	500 pg	10 µl
Wild-type DNA	50 pg/µl	500 pg	10 µl
Primer A	10 pmol/µl	400 pmol	40 µl
Primer B	10 pmol/µl	400 pmol	40 µl
2x SSCP Gel Loading Dye	2x		1 ml

Sequence of Primer A:	5'- GGG CTG GGC ATA AAA GTC A -3'
Sequence of Primer B:	5'- AAT AGA CCA ATA GGC AGA G -3'
2x SSCP Gel Loading Dye:	95% Formamide, 20 mM EDTA pH 8.0, 0.05% Xylene Cyanol, and 0.05% Bromophenol Blue

1.2 Additional Supplies Required

Taq DNA polymerase enzyme

10x *Taq* polymerase buffer

2.5 mM dNTPs

Sterile water

Thin-walled microfuge tubes - 200 µl or 500 µl size

Sterile aerosol tips

1.3 Storage Conditions

All kit components should be stored at -20 °C. The shelf life of the kit stored at -20 °C is 1 year.

Section 2
PCR Reaction

Note: It is extremely important that solutions and materials used during PCR set-up are not exposed to amplified DNA to avoid contamination during amplification.

1. Add the components listed below to 200 µl or 500 µl thin-walled microfuge tubes and mix.

	Tube 1 Wild-type DNA	Tube 2 Mutant DNA
Control DNA	1.5 µl	1.5 µl
Primer A	2.5 µl	2.5 µl
Primer B	2.5 µl	2.5 µl
10x *Taq* polymerase buffer	10.0 µl	10.0 µl
2.5 mM dNTPs	8.0 µl	8.0 µl
Taq DNA polymerase enzyme (5 U/µl)	0.5 µl	0.5 µl
Sterile water	75.0 µl	75.0 µl
Total volume	100.0 µl	100.0 µl

Note: 10x *Taq* polymerase buffer contains 100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, and 0.01% gelatin. If desired, add a third tube which contains no control DNA as a negative control.

2. Place the tubes into a thermocycler and enter the following parameters.

Step 1	94 °C	2 minutes	x 1 cycle
Step 2	94 °C	45 seconds	x 35 cycles
	61 °C	45 seconds	
	72 °C	45 seconds	
Step 3	72 °C	10 minutes	x 1 cycle

3. To check the product, add 5 µl of the amplified DNA to gel loading buffer and run on a 10% acrylamide/bis gel (10% Ready Gel—catalog number 161-0905). Run a DNA size standard, such as Bio-Rad's

100 bp Molecular Ruler (catalog number 170-8202), on the gel to approximate the size of the product. The size of the product should be 299 base pairs in length.

4. Store the amplified DNA at 4 °C. For long term storage (> 2 weeks), store the amplified DNA at -20 °C.

Section 3
SSCP Gel

In SSCP, a nondenaturing polyacrylamide gel is used to separate single-stranded DNA. Altered conformation due to a mutation in the sequence can cause the mutant single-stranded DNA to migrate differently than the wild-type DNA. This migration difference can be seen as a band shift between the mutant and wild-type DNA samples.

Note: Refer to the DCode universal mutation detection system manual for information on correct casting and running an SSCP gel on the DCode Universal Mutation Detection system.

1. Add 5 µl of the amplified mutant DNA and 5 µl of 2x SSCP gel loading dye to a microfuge tube. Gently mix the contents of the tube. Do the same for the amplified wild-type DNA.
2. Place the tubes (mutant and wild-type) into a 95 °C water bath for 7 minutes and then on ice for about 5 minutes.
3. Load 10 µl of the samples into the wells of an 8% acrylamide/bis gel (37.5:1), containing 7% glycerol, and 1x TBE buffer.

Note: Recipe for a 20 x 20 x 0.1 cm gel format.

40% Acrylamide/bis (37.5:1)	8 ml
10x TBE	4 ml
100% Glycercol	2.8 ml
TEMED	40 µl
10% Ammonium persulfate	400 µl
dH_2O	24.8 ml

4. Run the gel under the conditions listed below.

Buffer	1x TBE
Constant power	30 W
Buffer temperature	10 °C
Run time	3.5 hours

Note: Pre-chill the running buffer prior to a run.

5. After the run is completed, the gel can be stained with ethidium bromide (1 µg/ml) in 1x TBE buffer solution for about 5 minutes or SyBr Green II (Molecular Probes—1:10,000 dilution) in 1x TBE buffer solution for about 30–45 minutes. Visualize and photograph the gel by placing it on a UV transilluminator (Gel Documentation System 1000, catalog numbers 170-7520 through 170-7527, or Bio-Rad Polaroid Gel Documentation System, catalog numbers 170-3742 through 170-3749).
6. The result from your gel should look similar to that in Figure 1. There should be a band shift between the single-strands of the mutant and wild-type fragments.

Fig. 1. Separation of mutant and wild-type DNA on the DCode system. The samples were electrophoresed on an 8% acrylamide/bis gel containing 7% glycerol for 3.5 hours at 30 W with the buffer chilled at 10 °C. The gel was stained with SyBr Green II. Lane 1 contains the mutant DNA and lane 2 contains the wild-type DNA.

Section 4
Troubleshooting

Refer to the DCode universal mutation detection system manual for more troubleshooting details.

4.1 PCR

Problem	Cause	Solution
Faint, visible bands	Unused primers	1. A 19 bp fragment is not a problem.
	Concatemer DNA	2. Amplified DNA forms concatemer size DNA. This is not a problem when the sample is denatured for the SSCP gel.
No 299 bp fragment	No DNA	1. Make sure DNA is added to PCR reaction.
	Inactive enzyme	2. Use viable *Taq* DNA polymerase.
	Missing or bad dNTPs	3. Make sure all four dNTPs are used in PCR reaction.
	Missing primer/primers	4. Make sure both primers are used in PCR reaction.
Numerous bands	Nonspecific priming	1. Do set-up in PCR designated area.
		2. Make sure not to cross contaminate the mutant and wild-type DNA.

4.2 SSCP Gel

Problem	Cause	Solution
Streaking or DNA spikes in gel	Impurities in acrylamide	1. Filter before use. Check shelf life date of acrylamide solution.
		2. Be careful not to pierce wells while loading samples.
No difference in band shift	Incorrect running conditions.	1. Insure that the gel contains 7% glycerol.
		2. Run the gel at 10 °C.
		3. Denature samples prior to loading.

Section 5 References

1. Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. and Sekiya, T, *Proc. Natl. Acad. Sci. USA,* **86**, 2766-2770 (1989).